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Original paper

A physico-chemical characterization of oil from Camelina sativa seeds grown in Romania

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Abstract

It is well-known that camelina oil, obtained from *Camelina sativa* seeds is rich in fatty acids, predominantly linolenic acid and is used successfully in pharmaceutical and cosmetic therapies. It is remarkable that this species has a great adaptability to environmental conditions, being able to cultivate it on agricultural parcels and polluted soils.

The goal of this work is to evaluate the quantitative and qualitative differences of three types of camelina oils, obtained from varieties grown in Romania: GP 202-Spanish provenance, *Camelia* and *Mădălina*, both Romanian provenance. The effect of soil fertilization on the quality of the seeds /oil obtained from 3 varieties of camelina was tested, the control being the unfertilized *Camelina* variety. The following parameters have been evaluated: seed moisture, cold oil extraction yield, certain physico-chemical oil indicators and percent quantitative composition of individual and total fatty acids. Remarkable is the total fatty acid content of the oil that varies between 75.87 g% for the unfertilized *Mădălina* variant and 81 g% for the fertilized GP-202 variant. Taking in consideration the results for all the studied parameters, it can be concluded that the *Mădălina* variety is very similar to the other two, and fertilization contributes insignificantly to the improvement of the quality of the obtained oil.

Keywords *Camelina sativa*, oil extraction yield, fatty acids, linolenic acid, γ-tocopherol.

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Introduction

Camelina sativa is an oil seed plant belonging to *Brassicaceae* family, important due to*the high value oil obtained from its seeds* and *low input requirements* of the crop (VOLLMANN & EYNCK [1]).

The major part of camelina oil composition consists of unsaturated fatty acids, predominantly: linolenic acid (omega-3 fatty acid) – 28.0-50.3%, linoleic acid (omega-6 fatty acid) – 16.0-22.4%, oleic acid – 14.9-18.7%, eicosenoic acid – 11.6-17.5%. Compared to other oils obtained from cruciferous species, which contain significant amounts of erucic acid, camelina oil has the advantage of low content of this component (1.6-4.2%), which leads to reduced risks of cardiac damage in case of sustained administration (SHUKLA & al [2]; ABRAMOVIČ & ABRAM, [3]; MOSER & al [4]; JURCOANE & al [5]; TONCEA & al [6]; KATAR [7]; BELAYNEH & al [8]). Beside the fatty acid content, there has been reported by different researchers, a total content of tocopherols of 634-780 mg/kg (BELAYNEH & al [8]).

The linoleic and linolenic acids found in camelina oil have some interesting effects following topical application, as: tissue regeneration, involvement in membrane lipid transport and protective effect against chemical and enzymatic agents (ZHENG [9]; MENIS FERREIRA [10]), maintenance of the stratum corneum permeability barrier (MCCUSKER [11]), or lightning effect on the skin (ANDO & al [12]).

Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) compounds resulted from the metabolization of linolenic acid are involved in reduction of skin thickening and inhibition of collagen decrease after exposure to UV light (inhibition of MMP-1 and MMP-9), increasing expression of collagen fibers and elastic fibers (stimulation of TGF-β expression) (KIM [13]), reducing inflammation resulted after UV exposure (inhibition of erythema induced by UVB) (PUGLIA & al [14]). Gamma-tocopherol prevents solar burns (erythema, inflammation, edema) if applied consecutively to UV exposure, prevents edema and inflammation formation when applied before UV exposure, (YASUOKA [15]). The antioxidant effect (SIVAMANI & al [16]) and anti-inflammatory activity (YOSHIDA [17]) have also been demonstrated.

On the other hand, camelina crop has some agro-economic advantages as:

- High adaptability to climate conditions, being able to grow in temperate climate, semi-arid environments, and short season regions (EHRENSING & GUY [18]);
- Potential to grow on marginal lands, with a low quality soil and in areas with a high degree of pollution – for example CopșaMică an intenselyexploited village from Romania due to its methane gas deposits (DOBRE & al [19]);
- Increased resistance to weeds and pests;
- Short life cycle (85-105 days) which allows cultivation in rotation with other crops;
- There are developed environmental friendly technologies for the obtaining of camelina oil as cold mechanical pressing or supercritical CO_2 extraction.

Considering the therapeutic effects of camelina oil biocomponents, the existence of extraction technologies with valuable yields and minimal impact on the environment, as well as the agronomic and economic advantages of culture, camelina oil is a feasible choice for use as both the main active ingredient and in combination with other natural or synthetic compounds for pharmaceutical and/or cosmetic products with targeted action at cutaneous level.

The study presents a comparative analysis of three *Camelina sativa* varieties: *GP202*, *Camelia* and *Mădălina* with the aim of characterizing them in order to establish the more advantageous variety regarding the oil extraction yield and composition for future use in cosmetics and/or pharmaceutical products.

Materials and Methods

1. Materials

The varieties of camelina used were:

– *GP202*-Spanish variety, cultivated and harvested at Moara Domnească Teaching Farm;
– *Camelia*-eco-certified Romanian variety, seeds purchased from NARDI (National Agricultural Research and Development Institute) Fundulea;
– *Mădălina*-Romanian variety, also cultivated at Moara Domnească Teaching Farm.

For the last one (Mădălina variety), two cultivation technologies were used: with and without application of soil fertilizers (the future plan being to produce the seed in an ecological system).

The oil was obtained after mechanical cold pressing using a vegetal oil extraction equipment called IEU-00 from the National Institute of Research–Development for Machines and Installations Designed to Agriculture and Food Industry-INMA Bucharest. Before pressing, the seeds were conditioned for removal of impurities using the equipment ICS from the same Institute. The oil obtained was left at room temperature for decantation for 48 h. Following this step, the clear oil was separated from the sediment.

2. Determination of moisture content in camelina seeds

The camelina seeds moisture content was assessed using method 2.2.32-Loss on drying from the Ph. Eur. 9.0 [20].

3. Determination of oil extraction yield

70 kg of each type of camelina seeds were pressed using the IEU-00 equipment. The process lasted on average 30 minutes by charge. The oil extraction yield was determined by using the following formula:

$$\eta = \frac{m_{oil}}{m_{seeds}} \cdot 100$$

m_{oil} −quantity of oil resulted from the pressing process;
m_{seeds} −quantity of seeds subjected to pressing.

4. Determination of physical and chemical characteristics

All the determinations were done according with the European Pharmacopoeia following the methods from the table:

Nr	Parameter	Associated method from European Pharmacopoeia (9.0 Edition) [20]
1	Clarity and degree of opalescence; degree of coloration	-
2	Loss on dying	2.2.32
3	Odor	2.3.4
4	Peroxid value	2.5.5.
5	Acid value	2.5.1.
6	Refractive index	2.2.6.
7	Relative density	2.2.5.
8	Saponification value	2.5.6.
9	Iodine value	2.5.4.

5. Determination of fatty acids content in camelina oil

The fatty acid composition was determined using a GC-MS-MS (TRACE 1310/TSQ 8000 Evo) equipment-with a TG-5SILMS column (30 m x 0.25 mm x 0.25 μm) and helium as carrier gas. It was used an injection volume of 0.5 μl, a split rate of 1:100 and a solvent delay of 3 min. The temperature in the injector was 250ºC. The oven temperature program started at 170ºC for 2 minutes, and after two successive ramps it reached 240ºC, where it stood for 14 minutes. In the MS transfer line the temperature was 280ºC, while in the ionization source a 200ºC value was reached.

Identification and quantification of fatty acids was carried out by esterification in two steps. First, the oil samples were treated with methanolic NaOH solution (0.5 ml of 0.5 M concentration), 5 ml methanol and 5 ml hexane. The reaction has been achieved in the GC headspace termostat, at 70ºC, for 15 min. The second treatment applied to the samples consisted in addition of 1 ml of 14% metanolic BF3 solution and completing the esterification, under the same temperature and shaking conditions, for 5 minutes. The quantification of fatty acids was done using a certified mix solution of methylated fatty acids purchased from LCG (product code LA 90-1275). Except methyl oleate and methyl linolenate, for which the signal identification was done selectively, using the characteristic ions (fragment 292 for methyl linolenate and fragment 264 for methyl oleate), the other compounds were calibrated using usual total ions scaning.

6. Determination of tocopherols

γ-tocoferol identification was done using a HPLC Agilent Technologies 1260 coupled with a DAD detection system; the chromatographic column used was Poroshell 120 EC-C18, 50 x 3 mm, 2.7 μm. The thermostat for the column was fixed at 30ºC, and the mobile phase, a methanol: phosphoric acidsolution (0,1%) 95:5, had a flow rate of 0.5 ml/min; the injection was 10 μl. The reference solution, represented by γ-tocoferol in methanol-approximately concentration 100 μg/ml, was injected minimally 2 times before the samples. The acquisition time was 15 min and the detection was done at λ=280 (4)nm. Acquisition and processing of data were performed with an OpenLab/ChemStation software.

Results and Discussion

1. Determination of moisture content in *Camelina seeds*

Table 1. Values of loss on drying for camelina seeds

Camelina variety	Loss on drying (%)
GP 202	3.93
Camelia	3.83
Mădălina – with fertilizers	3.37
Mădălina – without fertilizers	4.24

The water content of all camelina seeds samples was similar and was well below the maximum seeds admitted humidity of 11% (FAN & ESKIN [21]). The highest value – 4.24%, registered for Mădălina – without fertilizers correlates with the fact that it was the last one to be harvested. For this sample there was a period of approximately 20 days between the harvest time and humidity assessment.

2. Determination of oil extraction yield

The camelina oil extraction from the seeds may be done with different methods by yield, conditions, equipment used. Researchers have tried to optimize the extraction of camelina oil and also to find the weaknesses and strengths of each of them.

CHANTSALNYAM & al [22] obtained an extraction yield of 38.52% using Soxhlet equipment for 8 h with n-hexane as solvent. MOSER & al [4] have obtained a yield of 30.5 wt% by Soxhlet extraction with hexane for 24 h. BELAYNEH & al [8] have made a comparison between

different extraction methods, obtaining the following results: classical Soxhlet extraction with hexane for 6 h – 35.9%, extraction by cold pressing – 29.9% and supercritical CO_2 extraction – 31.6%. MOSLAVAC & al [23] optimized the cold pressing extraction, establishing as optimal conditions a temperature of 52°C, a frequency of 20 Hz and a nozzle size of 9 mm. When setting these parameters, 289.5 ml of oil with 32.6°C temperature were obtained from 1 kg of camelina seeds.

Table 2. Extraction yield for different varieties of camelina

Camelina variety	m_{oil}	Extraction yield (%)
GP 202	22.02	31.46
Camelia	23.80	34.00
Mădălina - with fertilizers	22.24	31.77
Mădălina - without fertilizers	21.50	30.71

The extraction yields obtained by us, using cold mechanical pressing, are comparable with the ones presented by other researchers.

3. Determination of physical and chemical characteristics

The values for the assessed parameters are presented in Table 3.Considering that camelina oil is a relatively new ingredient in the cosmetic and pharmaceutical products there hasn't yet been introduced a monograph of it, neither in the European Pharmacopeia, nor in the USP. As a result, in order to have an image over the quality of the oil obtained, we believe that a comparison with the standards for edible oils and with the previsions of European Pharmacopoeia for similar vegetable oils is reasonable.

The oil obtained from all 4 samples seed was a clear liquid, with plant specific odor and color from yellow-Mădălina – without fertilizers to yellow greenish-for the other 3 samples.

The iodine value, the saponification value, the relative density and the peroxide value are important chemical parameters for the monitoring of oil quality. The highest peroxide value was obtained for Camelia variety – 3.07 meq/kg, while the lowest was quantified for Mădălina – without fertilizers – 0.95 meq/kg, values below the maximum acceptable limit specified by different worldwide standards. Peroxide value is an indicator of the quality and stability of oils, being a measure of the extent to which rancidity reactions have occurred. CODEX STAN 210 [24] specifies, for a series of edible vegetable oils, as acceptable limit a peroxide value of maximum 15 meq/kg oil for cold pressed and virgin oils. Also the SON (Standard Organization of Nigeria) (2000) and the NIS (Nigerian Industrial Standard) (1992) specify a standard value of 10 meq/kg (ZAHIR & al [25]).

Table 3. Physico-chemical characteristics of camelina

Camelina variety	Clarity	Color	Odor	Peroxide value, meg/kg	Acid value, mg KOH/g	Refractive index, 20°C	Relative density, 20°C g/cm^3	Saponification value, mg KOH/g	Iodine value, g I/100 g
GP 202	Clear liquid	Yellow-grenish	Plant specific	1.79	4.61	1.4772	0.9221	182.09	158.32
Camelia	Clear liquid	Yellow-grenish	Plant specific	3.07	3.94	1.4775	0.9220	178.60	143.18
Mădălina (with fertilizers)	Clear liquid	Yellow-grenish	Plant specific	2.96	0.63	1.4778	0.9227	184.07	162.26
Mădălina (without fertilizers)	Clear liquid	Yellow	Plant specific	0.95	1.72	1.4772	0.9200	183.33	156.57

Regarding the acid value, it was determined a value of only 0.63 mg KOH/g for Mădălina – with fertilizers, compared with 4.61 mg KOH/g for GP 202 variety. The refractive indexes determined at 20°C are similar, with no more than 0.0006points difference between the hightest (-1.4778) and the lowest value (-1.4772). These values are slightly higher than the ones mentioned in the Ullmann's encyclopedia of industrial chemistry, (1995) [26], for camelina oil-1.4698. The values of relative density, at 20°C were between 0.9200 g/cm^3 for Mădălina – without fertilizers and 0.9227g/cm^3 for Mădălina – with fertilizers, which falls in the interval of 0.9170-0.9240 g/cm^3 mentioned by KARLESKIND [27], for camelina oil relative density determined at 20°C.

For both, saponification and iodine value the highest result was obtained for Mădălina – with fertilizers (saponification value - 184.07 mg KOH/g, iodine value - 162.26 g I/100 g) while the lowest value was obtained for Camelia (saponification value - 178.60 mg KOH/g, iodine value - 143.18 g I/100 g). According with Ullmann's encyclopedia of industrial chemistry, (1995) [26] the iodine value for camelina oil is 127-155 gI/100 g and the saponification value is 180-190 mg KOH/g oil. The saponification value represents a measure of the average molecular mass of fatty acids in the oil, while the iodine value is a measure of the unsaturation in a vegetable oil. The lower the iodine value, the grater will be the oxidative storage stability (ZAHIR & al [25]).

Considering the overall characterization parameters, the results obtained by us are comparable with the onesdetermined by ABRAMOVIČ & ABRAM [3] on *Camelina sativa* oil from Slovenia: density at 20°C – 0.9207±0.0001 g/cm^3, refractive index at 25°C – 1.4756± 0.0001, peroxide value – 2.38±0.01 meq O_2/kg, acid value – 6.2 ±0.1.

Even if at this moment there is no monograph for camelina oil, we noticed that the acid value, theperoxide value, the iodine value and the saponification value obtained by us when characterizing the camelina oil are comparable with the ones specified in the European Pharmacopoeia monograph for the virgin linseed oil which has a fatty acids profile similar with the one of camelina oil, except the linolenic acid higher content. Considering that the above mentioned parameters depend on the unsaturation degree of the oil, we believe that they can be used as a guidefor camelina oil values: acid value – maximum 4.5 and peroxide value – maximum 15, iodine value 160-200, saponification value 188-195. (PH. EUR. 07/2014:1908 [20])

4. Determination of fatty acid composition

Camelina sativa oil is known as being polyunsaturated fatty acids rich. It has high omega-3 content, namely linolenic acid, compared with other vegetable oils: canola oil – 11.1%, rapeseed oil – 10%, soybean oil – 6.8%, corn oil – 1% (EIDHIN & al [28]).

The results obtained by our group of researchers are comparable with the ones found in literature:linolenic acid 28.0%-50.3%, linoleic acid – 15.2%-22.4%, oleic acid – 14.9-18.7%, eicosenoic – 11.6%-17.5%, erucic acid – 1.6%-4.2% (SHUKLA & al [2]; ABRAMOVIČ & ABRAM [3]; MOSER & al [4]; JURCOANE & al [5]; FAN & ESKIN [21]; TONCEA & al [6]; KATAR [7]; BELAYNEH & al [8]). FAN & ESKIN [21] reported also values for the following weaker represented acids, in the fatty acids profile: palmitic acid – 5.4%, stearic acid – 2.5%, eicosadienoic acid – 1.9%, palmitoleic acid – 0.1%, eicosanoic acid – 1.3%, behenic acid – 0.3% myristic acid – 0.1%.

DOMIL [29] reported the following values for camelina oil obtained from seeds cultivated in Romania-Banat region: palmitic acid (C16:0)-4.5 g/100 g oil, linoleic acid (C18:2)-16.3 g/100 g oil, linolenic acid (C18:3)-17.5 g/100 g oil, oleic acid (C18:1)-14.0 g/100 g oil, stearic acid (C18:0)-1.85 g/100 g oil.

The highest content of fatty acids was found in the oil of GP202 variety – 80.991%, followed by 80.680% in Mădălina – with fertilizers, 76.325% in Camelia and 75.867% in Mădălina -without fertilizers. The components distribution and total percentages for all oil samplesare detailed in Table 4.

5. Identification of γ-tocoferol

The only type of tocopherol identified by us in the camelina oil samples was γ-tocoferol. This was identified also in the camelina oil samples analyzed by us. It was identified by many researchers as being the major tocopherol from camelina oil (ABRAMOVIČ & ABRAM [3], SAGA & al [30]). The values are presented in Table 5.

Table 4. Fatty acids profile of camelina oil

	Camelina variety			
Fatty acid ester	**GP-202**	**Camelia**	**Mădălina - with fertilizers**	**Mădălina - without fertilizers**
Metilmiristat	0.041	0.039	0.041	0.037
Metilpalmitoleat	0.070	0.066	0.059	0.070
Metilpalmitat	4.580	4.180	4.260	4.240
Metil linoleate-C18:2	16.140	14.990	15.330	15.810
Metillinolenat -C18:3	31.520	30.100	32.870	27.050
Metil oleat-C18:1	11.150	10.440	10.830	10.960
Metilstearat	1.910	1.740	1.830	1.860
Metileicosenoat	14.050	13.360	14.090	14.040
Metil eicosanoat-C20:1	1.320	1.210	1.190	1.520
Metilbehenat	0.210	0.200	0.180	0.280
Total content of fatty acid esters	80.991	76.325	80.68	75.867

Table 5. γ-tocoferol presence in camelina oil

	Camelina variety			
	GP-202	**Camelia**	**Mădălina- with fertilizers**	**Mădălina - without fertilizers**
γ-Tocoferol (mg%)	47.6	46.2	40.0	46.6

The γ-tocoferol identified in the samples, had slightly different percentages ranging from 40.0% in Mădălina – with fertilizers sample to 47.6% in GP-202.

The tocopherols quantity reported by different researchersfor refined camelina oil, ranged between 580 mg/kg and 1564 mg/kg, while for unrefined camelina oil the content ranged between 695 and 994 ppm (FAN & ESKIN [21]). BELAYNEH & al [8] found for total tocopherols content values between 634 and 780 mg/kg. Concerning the different types of tocopherols, determined in fresh oil, the literature research reveals values of 41±8 mg/kg α-tocoferol, 710 ± 19 mg/kg-γ-tocoferol, 12±3 mg/kg- δ-tocoferol. β-tocoferol and tocotrienols were not identified (ABRAMOVIČ & al [31]).

Conclusion

The analysis of the oil extracted from the three Camelina varieties leads to the conclusion that the samples are highly similar, both from the point of view of the type of extraction and the fatty acid profile. Furthermore, it was found that the use of fertilizers does not result in significant quantitative or qualitative changes in the oils, all falling into the physico-chemical parameters specified by the European Pharmacopoeia for virgin linseed oil. Finally, considering the small differences between the Romanian and Spanish Camelina varieties, it is a feasible option to use oil from seeds harvested in environmentally friendly conditions for future studies.

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